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FULL PAPER

Harnessing coumarin chemistry: Antibacterial, antifungal, and antioxidant profiling of novel coumarin derivatives

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The rational design of 4-hydroxycoumarin derivatives is crucial due to their therapeutic significance and structural versatility. This study focused on synthesizing and characterizing various coumarin derivatives using spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR), Nuclear magnetic resonance spectroscopy (NMR), and elemental analysis CHN. The antibacterial and antifungal activities of these derivatives were assessed against specific bacterial and fungal strains, respectively. The results revealed strong microbiological activity with, inhibition zones ranging from 6 to 27 mm against bacteria and inhibition percentages ranging from 6% to 26% against fungi. Furthermoe, the compounds exhibited significant antioxidant properties with scavenging percentages ranging from 27% to 91% as determined by the DPPH radical scavenging assay. These findings underscore the potential of coumarin derivatives as versatile therapeutic agents with promising applications.

KEYWORDS

Coumarin derivatives; antibacterial activity; antifungal activity; antioxidant activity; green chemistry.

Introduction

The class of compounds called coumarins has garnered significant interest in medicinal chemistry due to the diverse range of biological activities and adaptability in structure [1-3]. Among these, 4-hydroxycoumarin derivatives stand out for the notable pharmacological properties, including antibacterial, antifungal, and antioxidant action, making them promising candidates for therapeutic applications [4-8]. With the increasing prevalence of antibiotic resistance, there is pressing need for novel drug, candidates and coumarin derivatives offer potential solutions. Modern drug discovery strategies emphasize the design and synthesis of coumarin derivatives with specific biological activities, taking into account the urgent need for environmentally friendly drug candidates. Coumarins exhibit a wide array of pharmacological effects,

including antibacterial, anti-inflammatory, and anti-cancer properties, making them crucial subjects of clinical research. Furthermore, their structural diversity allows for various chemical modifications to enhance their biological features [9-13]. The synthesis of coumarin derivatives using green chemistry principles aligns with global efforts to promote sustainable drug development technologies. In this work, we aim to efficiently synthesize and characterize new 4-hydroxycoumarin derivatives while emphasizing environmentally friendly procedures. Structural characterization will involve elemental analysis along with spectroscopic techniques such as nuclear magnetic resonance NMR spectroscopy and fourier transform infrared FTIR spectroscopy [14-21]. Following synthesis and characterization, we will evaluate the antibacterial, antifungal, and antioxidant properties of the newly synthesized coumarin derivatives against oxidative stress models and clinically relevant microbial strains. Through systematic investigation our research aims to contribute to the field of medicinal chemistry by expanding the knowledge base and accelerating the development of innovative therapeutic agents with high efficacy and reduced environmental impact. The novel aspects of this research lie in the innovation methods employed for synthesizing and evaluating the coumarin derivatives. Specially novel senthyic pathways where developed to create these derivatives, incorporating unique chemical reactions and modification. In addition, they the evaluation process involved comprehensive screening for antibacterial, antifungal and antioxidant properties providing holistic assessment of the compounds pharmacological potential. These innovation approach distinguish this study from previous research in the field of contribute to advancing our understanding of coumarin chemistry and its applications and a drug discovery. The specific objectives of this study are as follows:

1. Synthesize novel coumarin derivatives using innovative synthetic pathways and chemical modifications.
2. Characterize the synthesized compounds using spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, and CHN elemental analysis.
3. Evaluate the antibacterial properties of the synthesized coumarin derivatives against a panel of Gram-positive and Gram-negative bacterial strains.
4. Assess the antifungal activity of the coumarin derivatives against common fungal pathogens, including *Aspergillus niger* and *Candida albicans*.
5. Determine the antioxidant properties of the synthesized compounds using the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.
6. Compare the efficacy of the synthesized coumarin derivatives with existing standards and assess their potential as multifunctional drugs for therapeutic applications.
7. Contribute to the body of knowledge in coumarin chemistry and drug discovery by elucidating the structure-activity relationships and pharmacological potential of the synthesized compounds.

Experimental

General

The chemicals utilized in the manufacturing process we are supplied by Sigma-Aldridge (Selangor, Malaysia). Infrared (IR) Spectra were obtained using a thermos Nicolet Corp. Nicolet FTIR 6700 spectrophotometer based in Madison, WI, USA with values reported in centimeter^{-1}. Nuclear magnetic resonance (NMR) Spectra where record it recorded on an AVANCE III 600 MHz spectrometer, with DMSO serving as an internal standard for chemical shifts displayed in Sigma ppm. Elemental microanalysis was conducted using an elementar vario EI III Carlo Erba 1108

element analyzer from Carlo Erba the Reagenti SpA, located in Rodano Italy.

Coumarins synthesis

a. Synthesis of synthesis of methyl 2-(coumarin-4-yloxy)acetate

Bromoacetate methyl ester (18.3 mmol) and potassium carbonate (67.82 mmol) were reflexed with 4 hydroxy coumarin (12.34 mmol) and 30ML of acetone for 12 hours. After cooling, the mixture was evaporated to dryness, and the residue was partitioned between 50 mL of CHCl3 and 100 mL of water. The organic phase was dried using potassium disulfate, followed by filtration and evaporation to yield 85% of the product. Recrystallization from acetone yield a product with melting point between 84 and 85 degrees Celsius; ^{1}H-NMR ($CDCl_3$): δ 7.3111, 7.555, 7.896 (three s, 1H each , aromatic ring) 5.58 (s, 1H, -C = CH), 4.79 (s, 2H, CH_2), 3.6 (s, 3H, CH3); FTIR in cm^{-1}: 1,567.2 (C = C, aromatic), 1,624.5 (C = C), 1,723.1 (lac.), 1,760.3 (C=O), 3,083.4 (C-H, aromatic), and 2,960 (C-H), [22].

b. The production of 2-(coumarin-4-yloxy)acetohydrazide (2)

Compound 2 (2.34 g, 10 mmol) was refluxed in 25 mL of ethanol for 4 hours with 15 mmol of hydrazine hydrate. A 55% yield was obtained by recrystallizing the oily mass that separated after concentrating the reaction mixture with ethanol; ^{1}H-NMR (CDCl3): δ 8.21 (Q, 1H, NH), 7.41–7.78 (m, 4H, aromatic ring), 5.43 (s, 1H, -C = CH), 4.75 (s, 2H, NH_2), 4.45 (s, 2H, CH2); FTIR (cm^{-1}): , 1,624.2 (C = O), , 1,721.4 (C = O, lac.), 3,233.3, 3,210 (NH), , 3,083.9 (CH, aromatic), and 2,959.0 (CH, aliphatic) .

c. Schiff base synthesis 3-5

Compound 2 (0.2 mmol) was refluxed in 25 mL of ethanol for 20 h with either 2-methylbenzaldehyde, 4-aminoantipyrene, or 0.2 mmol ethyl methyl ketone. The solid mass was filtered and recrystallized from ethanol after cooling to room temperature.

2-(Coumarin-4-yloxy)acetohydrazide, *N*'-(2-methylbenzylidene) (3). 50% return. ^{1}H-NMR (CDCl3): δ 2.891 (s, 3H, CH3), 4.19 (s, 2H, CH2), 5.355 (s, 2H, O-CH2), 5.57 (s, 1H, -C = C-H), 7.270 –7.75 (min, 4 h, aromatic ring); 8.31 (Q, 1H, N = CH), 8.01 (Q, 1H, NH); FTIR (cm^{-1}): 1,627.7 (C = C), 1,683.7 (C = O, amide), 3,199.3 (N-H), 1,730.3 (C = O, lac.), 3,067.2 (CH, aromatic), 2,965.4 (C-H, aliphatic) [22].

N'-(2-(coumarin-4-yloxy)acetohydrazide (4),4-amino-1,5-dimethyl-2-phenyl-1H-pyrazole-3(2H)-ylidene. 60% yield; MP 162-163 °C, 1H-NMR (CDCl3): 4.67 (s, 2H, O-CH2), 4.05 (s, 2H), 5.23 (s, 1H, -C = CH), 2.83 (s, 3H, CH3), 3.21 (s, 3H , CH3), 7.11–7.34 (m, 4H, aromatic ring), 8.41 (s, 1H, N=CH), and 8.0 (s, 1H, NH); FTIR (cm^{-1}): 3,432.8 (NH2), 2,989.9 (C-H, aliphatic), 3,328.0 (N-H), 1,725.8 (lac.), 3,077.3 (C-H, aromatic), 1,651.9 (C=O, amide), 1.6229 (C = C), and 1,616.3 (C = N). CHN analysis: C 61.91 percentage, N 17.19 percentage, H 5.20 percentage, found: H 5.641 percentage, C 60.057 percentage, and N 16.685 percentage.

Coumarin-4-yloxy N'-Butane-2-ylidene)-2-acetohydrazide (5). deputy; 50% return. 71-72 °C; 1H-NMR (CDCl3): δ 0.81 (t, 3H, CH3), 1.49 (q, 2H, CH2), 2.01 (s, 3H, CH3), 4.64 (s, 2H, O-CH2), 5.21 (s, 1H, -C = CH), 6.95 (s, 1H, N = CH), 7.38-7.79 (m, 4H, aromatic ring); IR (cm−1): 3,198.2 (N-H), 2,922.9 (C-H, aliphatic), 3,068.4 (CH-H, aromatic), 1,732.2 (C=O, lactone), 1,673.1 (C=O, amide), 1,615.3 (C=N), 1621.5 (C=C); Analysis: C 62.49%, H 5.59%, N 9.72%. H 4.89%, N 9.11%, and C 61.70%

Pharmacological evaluation

a. Antibacterial assessment

In vitro antibacterial activity of the of the tested compounds was evaluated using the disk diffusion method [23] on nutrient agar

media against four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Proteus vulgaris*) and Gram-positive bacterium (*Staphylococcus aureus*). Bacterial culture were grown on agar medium and incubated for 24 hours at 37°. Sterile filter paper discs (diameter: 5 mm) impregnated with varying amounts (1-10 mg/disc) of coumarin derivatives dissolved in sterile dimethylsulfoxide (DMSO) where placed on agar plates inoculated with bacterial strains. After the specified incubation period, the inhibition zones around each disk were measured. Control studies using DMSO alone were conducted to assess any potential impact on biological activity, and no activity against the bacterial strains was observed. All experiments were repeated three times, and the average was calculated to ensure reproducibility and accuracy.

b. Antifungal activity assessment

antifungal activity was evaluated based on the inhibition of growth of *Aspergillus niger* and *Candida albicans* and potato dextrose broth (PDB) medium [24,25]. Spors suspensions (5 x 106 cfu/mL) of the tested fungi where added to medium in Erlenmeyer flasks under aseptic conditions. Various considerations (0.5-1.00 mg/mL) of the synthesized coumarins where added to the flasks, followed by incubation at 27 plus minus one centigrade in the dark for five days. The mycelium was then collected, dried and weighted. The inhibition level relative to the control was determined using Equation (1):

$$\text{Percentage of inhibition} = \frac{C - T}{C} \times 100 \quad (1)$$

Where, T is the weight of mycelium from the test flasks and C is the weight of mycelium from the control flasks.

c. Antioxidant activity assessment

The the antioxidant activity was evaluated using DPPH (2,2-diphenyl-piceylhydrazyl) radical scavenging essay. Sample solutions were prepared at various concentrations (200, 400, 600, 800, and 1000 if/mL) from a stock solution (1 mg/mL) and mixed with ethanolic DPPH solution (0.3 moles). The mixture was allowed to stand at room temperature for 30 minutes and the absorbance was measured. at 517 nanometer using UV-Visible spectroscopy. Lower absorbance values indicate Higher free radical Scavenging activity. Ethanol used as solvent and ascorbic acid served as standard the percentage of DPPH radical. All experiments were repeated three times, and the average was calculated to ensure reproducibility and accuracy. Scavenging activity was calculated using Equation (2):

$$\text{Scavenging effect}(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards.

Results and discussion

Our study is significant in the context of recent advancements in the field, where there is a growing interest in the synthesis and evaluation of novel compounds for various biomedical applications. Recent studies have underscored the potential of coumarin derivatives as promoting agents with the diverse pharmacological activities including antibacterial, antifungal, and antioxidant properties. However, gaps in the knowledge persist regarding the synthesis of coumarin derivatives with enhanced efficacy and specificity against microbial pathogens as well as their comprehensive characterization using advanced spectroscopic techniques. The current work addresses these gaps by employing innovating synthetic methodologies to generate a series of novel coumarin derivatives and rigorously evaluating their biological activities by synthesizing compounds with tailored structural features, we aim to optimize their pharmacological properties thereby

enhancing their potential as therapeutic agents. Furthermore, our study utilize combination of FT-IR, NME, and CHN analysis to comprehensive characterize the synthesized compounds.

This approach is crucial as allows us to gain insights into the molecular structure composition and functional groups presence in the compounds thus providing a thorough understanding of their chemical properties. By employing these advanced characterization techniques we aim to if elucidate thestructure-activity relationships of the coumarin derivatives and identify key molecular features that contribute to their biological activities.

Chemistry

The synthetic pathway for generating coumarin derivative from 4-hydroxycoumarin Is Illustrated in Figure 1. Methyl 2-(coumarin-4-yloxy)acetate (1) was synthesized by refluxing methyl bromoacetate with 4-hydroxycoumarin and anhydrous acetone facilitated by anhydrous potassium carbonate. In the FT-IR spectra of compound 1, characteristic absorption bands at 1,760 cm^{-1} and 17,23.1 cm^{-1} corresponded to the carbonyl groups of ester and lactone, respectively. The ^{1}H-NMR spectrum displayed peaks at sigma 4.79 ppm for methylene group, 3.6 ppm for three protons of methyl group, and 5.58 ppm for allylic proton. The synthesis of 2-(coumarin-4-yloxy)acetonhydrazide was achieved by reacting compound one with hydrazine hydrate compound to exhibited characteristic of absorption bonds hot 3,233.3 cm^{-1} and 3,210.0 cm^{-1} in FT-IR Spectrum corresponding to the hydrazide groups and carbonyl bonds at 1,721.4 cm^{-1} and 1,624.2 cm^{-1} for lacton and amide, respectively. The NMR Spectrum displayed a singlet at Sigma 8.21 ppm for the signal and NH proton and singlet at 4.45 pm for the two methylene protons. Further reactions of compound 2 with various carbonyl compounds including 2-methylbenzaldehyde, 4-aminoantipyrine and ethyl methyl ketone resulted in the formation of *N*'-(2-methylbenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy)acetohydrazide (3), *N*'-(4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-ylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy)acetohydrazide (4) and *N*'-(butan-2-ylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy)acetohydrazide (5), respectively. Compound 3 is exhibited absorption bands at 1,683.7 cm^{-1} for carbonyl and 3,199.3 cm^{-1} for an Eno groups in the ftir Spectrum the NMR Spectrum short singlets at Sigma 2.891 PPM for the methyl protons, Sigma 4.19 ppm for the methylene group and 8.13 ppm for azomethane proton. Compound for displayed absorption bands at 1,725.8 cm^{-1} for a amide and 1,651.9 cm^{-1} for carbonyl in the FT-IR spectrum, along with absorption bands at 3,432.8 cm^{-1} and 3,328.0 cm^{-1} for amino groups. The proton NMR spectrum revealed singlets at Sigma 4.05 ppm and 4.67 ppm for two protons, while the methylene group exhabited bands at Sigma 5.23 ppm. Furthermore, singlets at Sigma 8.0 ppmand 8.41 ppm corresponding to the protons of amino group and azomethane, respectively. Compound 5 exhibited absorption bands at 3,198.2 cm^{-1}, 1,732.2 cm^{-1}, and 1673.1 cm^{-1} for amino, lactone, and I amide groups respectively, in the FT-IR spectrum along with an absorption band at 1615.3 cm^{-1} for the other group the animal Spectrum short singlet Sigma 6.95 ppm for the azomethane proton, a quartet at Sigma 1.49 ppm for methylene group and a triplet at Sigma 0.81 ppm methyl protons. Through comprehensive spectroscopic analysis the successful synthesize of these coumarin derivatives was confirmed providing valuable insights into their structural characteristics and paving the way for further pharmacological investigations. The characterization techniques serve complementary roles in characterizing chemical compounds. FTIR provides information about functional groups and molecular structures, NMR offers insights

into molecular composition and connectivity, and CHN elemental analysis quantifies elemental composition. Using all three techniques ensures comprehensive characterization, as each method provides unique data that collectively enhance understanding of the synthesized compounds' properties.

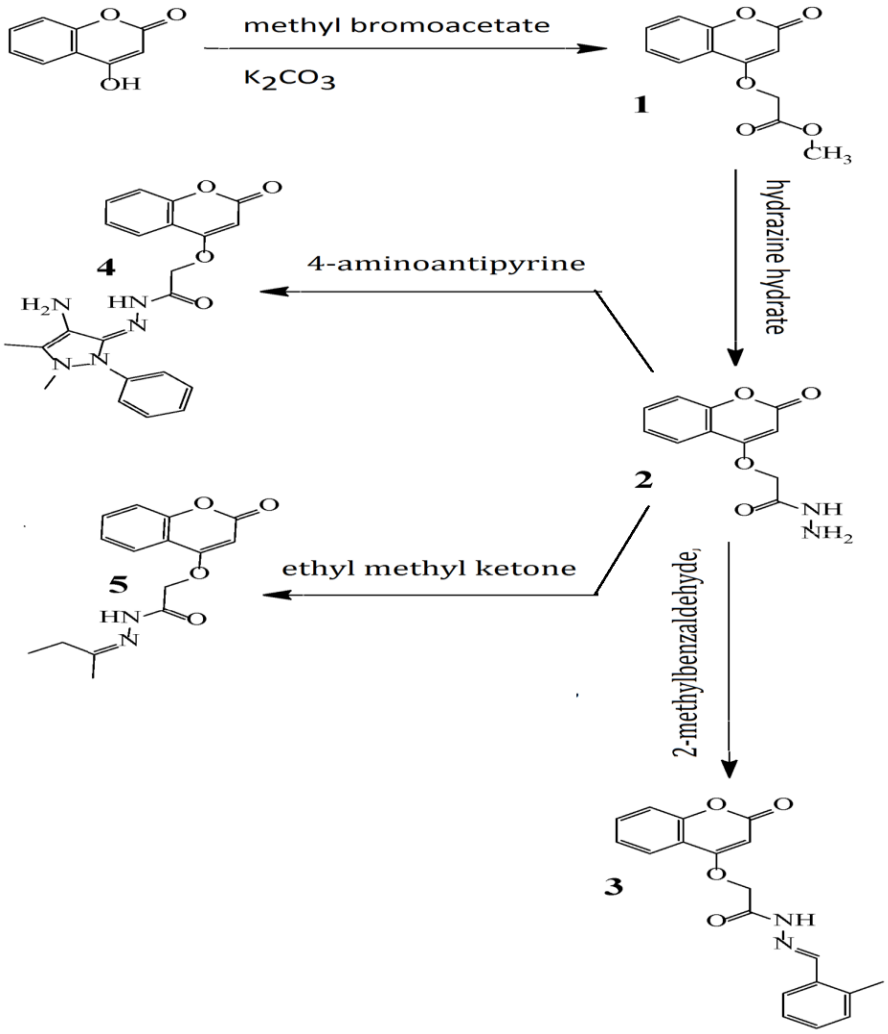

FIGURE 1 The synthesized coumarins

Pharmacological investigations

All experiments were performed using well-established protocols and standard procedures to ensure the reliability and accuracy of the results. Specifically, the antibacterial and antifungal activities of the manufactured coumarin derivatives were evaluated using standard microbiological assays, including identification of zones of inhibition against bacterial and fungal strains. These tests were performed in triplicate, and results were expressed as mean values ± standard deviation to account for variability and ensure repeatability.

In addition, the antioxidant properties of the compounds were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, which is a widely accepted method for evaluating antioxidant activity. The scavenging percentages were compared to standard antioxidants, such as ascorbic acid, to provide a reference point for observed radical scavenging activity.

a. *Antibacterial activities*

The evaluation of compounds 1 to 5 demonstrated significant antibacterial properties surpassing those of the parent

compound 4-hydroxycoumarin. The enhanced activity observed in the compounds 1 to 5 can be attributed to the incorporation of nitrogen atoms and azomethane groups. Azomethane groups are now for imparting for potent bactericidal activity resulting in more effective eradication of bacteria compared to the parent compound alone. Notably compound 1to 5 exhibit unpaired electrons and double bonds shared with donor atoms within the coumarin nuclei, suggesting potential pi-electrons delocalization throughout the compounds. This phenomena enhances lipophilic character of compounds 1 to 5, facilating their permeation through the lipoid layers of bacteria membrane. The increased lipophilicity of compound 1 to 5 likely contributes to their enhanced antibacterial efficacy. It is plausible that these compounds deactivate various cellular enzymes crucial for microorganisms metabolic pathways. Moreover, it is hypothesized that ultimate action of the toxicant involves the denaturation of one or more cellular proteins thereby disrupting normal cellular processes. From the antibacterial study of compound 1 to 5 (Figures 2-6), several conclusions can be drawn:

1- Overall, the synthesized compounds exhibited higher antibacterial activity against *E.coli* bacteria compared to other bacterial strains.

2- Compound for demonstrated superior activity against all tested bacteria followed by compound 3 in comparison to the other compounds the heightenated stability of the 4-aminoantipyrine moiety present in compound 4, coupled with efficient coupling reactions, likely contributes to the exceptional antibacterial activity. These findings underscore the potential of compounds 1 to 5 as a promising antibacterial agents, with compound 4 standing out as a particularly protent candidate warranting further investigation. The antibacterial activity of compounds 1 to 5 against selected bacterial strsins, including *Pseudomonas aeruginose*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Staphylococcus aureus*, where improved at various concenrations. The results reveal distinct concentration-dependent effects on bacterial growth inhibition for each compound.

In the assessment against each bacteria strain, compound 1exhabited moderate antibacterial activity. Notably, compound 1 demonstrated increased suppression of bacterial growth at higher concentrations (0.75 and 1) particularly against *Staphylococcus* and *E. coli*. However it is effectiveness against *Proteus vulgaris* and *Pseudomonase aeruginosa* with relatively lower. Compound 3 demonstrated significant antibacterial activity across all tested concentrations. Notably, it displayed remarkable inhibition of bacterial growth at dose of 0.5 and above, particularly against *E. coli* and *Staphylococcus aureus*. While it is effectiveness against *Proteus vulgaris* was slightly lower, it is still exhibited notably activity.

Compound 4 exhibited strong antibacterial activity against all bacterial strains tested. It demonstrated potent reduction in bacterial growth at concentrations ranging from 0.5 to 1 particularly against *E. coli Klebsiella* and Staphylococcus aureus. Although slightly less effective noteworthy activity against *Proteus vulgaris* and *Pseudomonas aeruginosa*. Compound 5 displayed antibacterial activity varying from mild to strong against all bacterial strains examined. Significant inhibition of bacterial growth was observed at concentration of 0.75 and 1, particularly against *Escherichia coli*, K*lebsiella pneumoniae,* and *Staphylococcus aureus*. Although slightly less effective, it also exhabited notably activity against *Proteus vulgaris* and *Pseudomonase aeruginosa*. Overall compound 4 demonstrated the highest efficacy at all doses while compounds 2, 3 and 5 generally exhibited the strongest antibacterial activity against the bacterial strains studied. These findings suggest the potential of this

compounds are effective antibacterial agents warranting further research and development to address bacterial diseases. It is possible to hypothesize the mechanism of action by which compound 1 to 5 kill bacteria based on structural properties and antibacterial qualities. Proposed mechanism of action:

1- Membrane distribution: Compounds 1 to 5 owing to their lipophilic nature and presumed to interact with the lipid bilayer of bacterial membranes. The presense of azomethan groups and nitrogen atoms enhances membrane permeability, potentially leading to membrane destabilization. This disruption may result in the leakage of cellular contents and eventual cell lysis.

2- Interference with cellular processes: Upon entry into bacterial cells, compounds 1through 5 have potential to interfere with essential biological processes. They may target key enzymes involved in bacterial metabolism or desrupt critical processes such as DNA replication and protein synthesis. This interference can lead to cellular dysfunction and eventual bacterial death.

3- Protein denaturation: The reactive functional groups presents in compounds 1 to 5 such as amino groups and others could facilitate the denaturation of bacterial proteins. Through interaction with amino acid residues these compounds may alert the structural integrity of proteins, impairing their functionality. Protein denaturation disrupts vital cellular processes contributing to bacterial mortality.

4- Generation of reactive oxygen species (ROS): Certain compounds with antioxidant properties, including coumarins, may induce antibacterial effect by triggering the production of reactive oxygen species within bacterial cells. ROS such as hydrogen peroxide and superoxide radicals can induce oxidative damage to cellular components including lipids proteins and DNA. This oxidative stress overwhelms bacterial defense mechanisms leading to Cellular injury and eventual cell death.

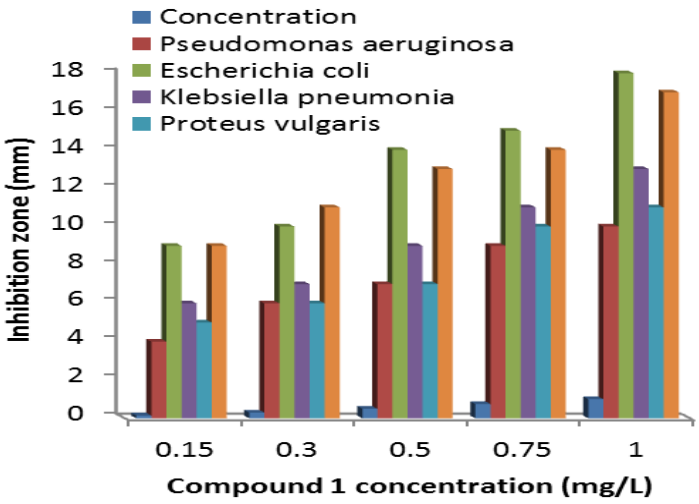

FIGURE 2 Inhibition activities of various concentration of compound 1 against selected types of bacteria

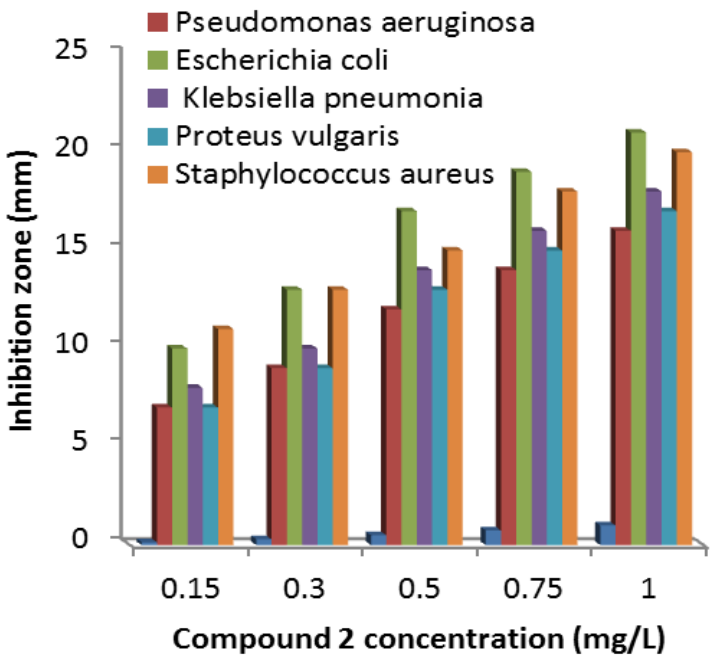

FIGURE 3 Inhibition activities of various concentrations of compound 2 against selected types of bacteria

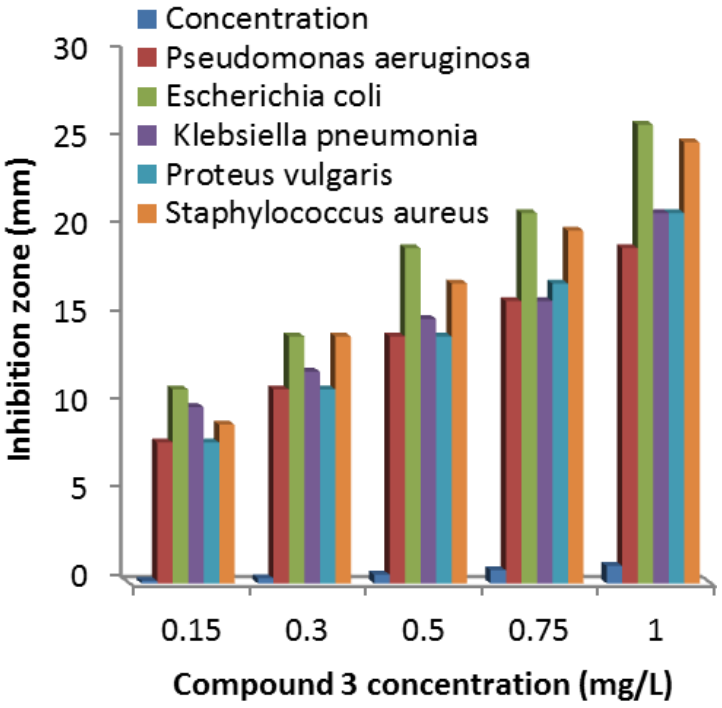

FIGURE 4 Inhibition activities of various concentrations of compound 3 against selected types of bacteria

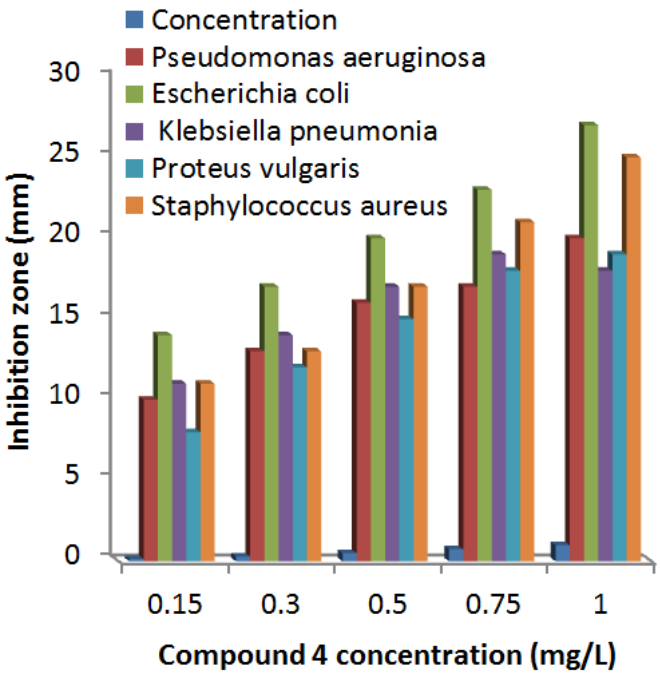

FIGURE 5 Inhibition activities of various concentrations of compound 4 against selected types of bacteria

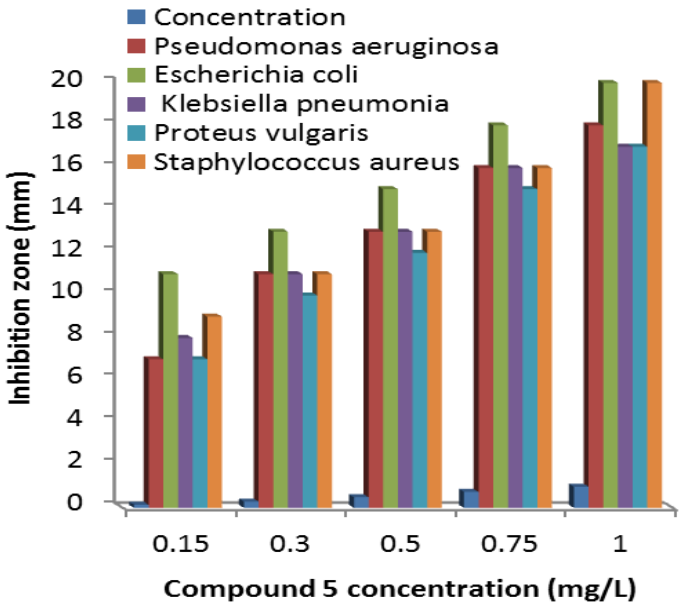

FIGURE 6 Inhibition activities of various concentrations of compound 5 against selected types of bacteria

b. Antifungal activities

The antifungal efficacy of compound 1 to 5 was assessed against notable fungal species including *Aspergillus niger* and *Candida albicant*. Among the compounds tested, compound 2 exhibited significant antifungal activity against all examined fungal strains as in Figures 7 and 8.

Several factors may contribute to the antifungal activities observed:

1- Structure features: Compounds such as compound 2 processes structure motifs,

including hydrazide and aromatic groups which could interact with the fungal cell components, disrupting essential cellular process

2- Hydrophobic interactions: Compounds with lipophilic properties like coumarin derivatives may engage in hydrophobic interactions with fungal cell membranes. This interaction can destabilize the membrane compromising the Integrity of the fungal cell wall and membrane.

3- Disruption of fungal cell membrane: Analogues to their antibacterial mechanism compounds 1 to 5 may disrupt fungal cell membranes leading to leakage of cellular contents and eventual cell death. This disruption may occur through direct interaction with membrane components or interference with membrane-associated proteins.

4- Inhibition of fungal growth: Compounds 1 to 5 may inhibit fungal growth by disrputing essential metabolic pathways or cellular processes crucial for fungal survival. This inhibition could target enzymes involved in fungal metabolism protein synthesis or cell wall synthesis impeding fungal growth and replication.

5- Generating of reactive oxygen species: Compound processing antioxidant properties may include antifungal effects by generating effective oxygen species with fungal cells. ROS can induce oxidative stress damaging cellular components and leading to fungal cell death.

Proposed mechanism for antifungal activities:

1- Membrane rapture: Compounds 1 to 5 interact with fungal cell membranes disrupting their integrity and causing leakage of cell contents.

2- Inhibition of cell wall formation: Some compounds may inhibit fungal cell wall formation weakening the structural integrity of the wall and compromising fungal viability.

3- Interference with cellular processes: Compounds may hinder essential enzymes or metabolic processes involved in fungal protein synthesis, DNA replication, or metabolism, therapy impending fungal growth and reproduction.

4- Induction of oxidative stress: Compounds with antioxidant properties can induce oxidative stress in fungal cells by generating reactive oxygen species leading to fungal cell death.

These proposed mechanisms highlight various pathways through which compounds 1 to 5 may exert their antifungal effects, underscoring their potential as therapeutic agents against fungal infections.

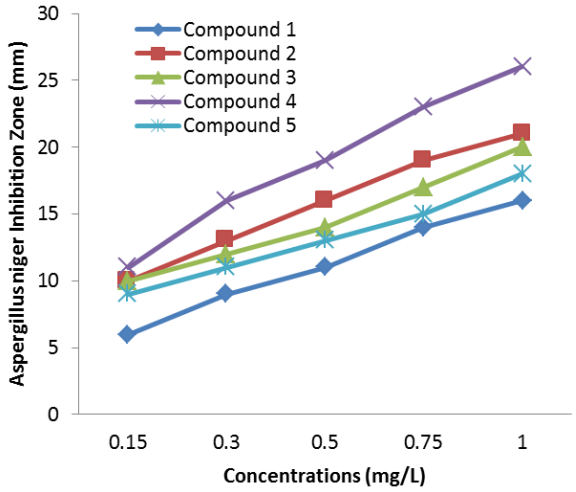

FIGURE 7 Inhibition activities of various concentrations of compounds 1 to 5 against Aspergillus niger

Compounds 1 to 5 where evaluated for their ability to inhibit the growth of *Aspergillus niger* at varying concentrations (0.15 mg/L, 0.3 mg/L, 0.5 mg/L, 0.75 mg/L, and 1.0 mg/L). The result elucidated in Figure 7 indicate a dose-dependent response, with a greater inhibition of fungal growth observed at higher concentrations of the compounds. Compound 1 exhibited modest inhibitory activity with a fungal growth inhibition Zone of 6 mm at the lowest tested concentration of 0.15 mg/L. As the concentration of compound 1 increased the inhibition of *Aspergillus niger* growth also increased reaching 16 mm at the highest concentration 1 mg/L. In comparison, compound 2 to demonstrated more potent inhibitory effects across all tested concentrations. At the lowest dose (0.15 mg/L) compound 2 reduced fungal growth by 10 mm while the highest concentration 1 mg/L the inhibition zone extended to 21 mm. Similarly compound 3 exhibited does-dependent suppression of a Aspergillus niger growth .The inhibition zone where 10 mm at 15 mg/L and 20 mm at 1 mg/L . Among all compounds tested compound 4 displayed the the strongest inhibitory action at the lowest concentration 0.15 mg/L. Compound 4 reduced fungal growth by 10 mm while at the highest concentration 1 mg/L the inhibition zone expanded to 26 mm. Compound 5 also showed dose-dependent suppersion of fungal growth with inhibition zones ranging from 9 mm at 0.15 mg/L to 18 mm at 1 mg/L. Compound 4 exhibited the highest potency among compounds 1 to 5 showcasting variying degrees of antifungal activity against *Aspergillus niger*. These finding underscore the potential of these compounds as fungal agents through further research is warranted to allucidate their mechanisms of action and optimize their efficacy in treating fungal infections.

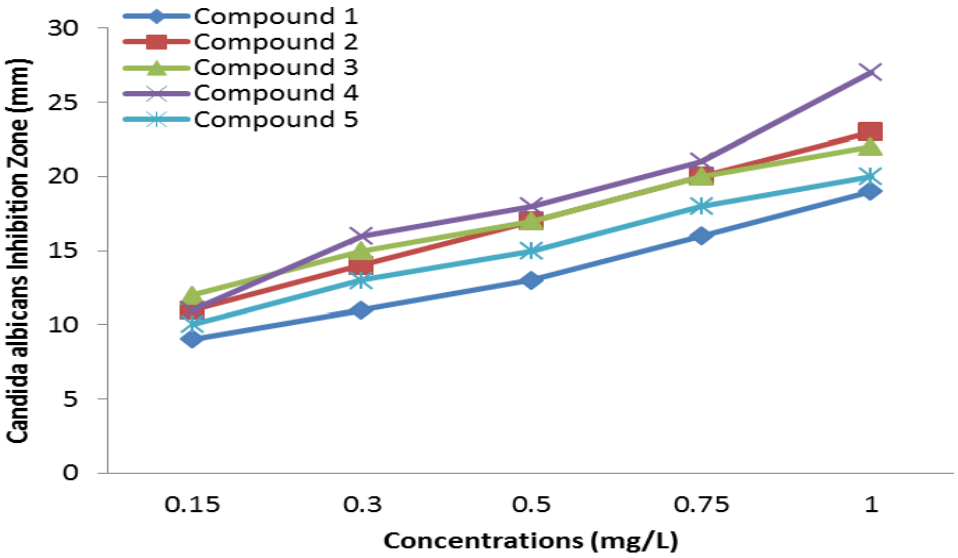

FIGURE 8 Inhibition activities of various concentrations of compounds 1 to 5 against Candida albicans

Compounds 1 to 4 were assessed for their ability to inhibit *Candidate albicans* at varying concentrations ranging from 0.15 mg/L to 1 mg/L. The findings depicated in Figure 8, reveal a concentration-dependent trend, with stronger inhibition of fungal growth observed at higher compound concentrations. Compound 1 exhibited moderate inhibitory activity against *Candidate albicans* at the lowest concentration tested (0.15 mg/L),

resulting and a 6 mm inhibition zone. Increasing the concentration of compound 1 led to enhancing inhibition of candidates growth reaching a maximum inhibition zone of 16 mm at 1 mg/L. In contrast, compound 2 demonstrated more potent inhibitory effects across all concentrations tested. At 0.15 mg/L compound 2 reduce fungal growth by 10 mm whereas at 1 mg/L the inhibition zone extended to 21 mm. Similarly compound 3 exhibited dose-dependent suppression of *Candida albicans* growth with inhibition zons of 10 mm at 0.15 mg/L and 20 mm at 1 mg/L. Among all compounds examined compound 4 displayed the strongest inhibitory effect at the lowest concentration 0.15 mg/L. Compound 4 inhibited fungal growth by 11 mm, while at 1 mg/L the inhibition zone increased to 26 mm. In addition, compound 5 demonstrated dose-dependent inhibition of fungal growth, with inhibition zones ranging from 9 mm at 1.5 mg/L to 18 mm at 1 mg/L. Compound 4 exhibited The highest potency among all compounds tested showcast varying degrees of antifungal activity against Candida albicans. These findings underscore the potential of these compounds as antifungal agents, highlighting the importance of further research to elucidate their mechanisms of action and optimize their efficacy for medical applications against Candida albicans infections.

c. *Antioxidant activity*

Compounds 1 to 5 were assist for their antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging essay at concentrations ranging from 200 µg/mL to 1000 µg/mL. The assay provides insights into the ability of antioxidants to neutralize free radicals and prevent oxidative damage. Compound concentrations where varied to observe dose-dependent responses and assess the kinetics of the reaction between antioxidants and DPPH radicals. The mechanism underlying the antioxidant activity of compounds 1 to 5 involves several pathways:

1- Structural properties:
Functional groups such as hydroxyl and hydrazide along with aromatic rings present in compounds 1 to 5, contribute to their antioxidant properties. These groups donate electrons to neutralize free radicals thereby reducing oxidative damage.
2- Hydrogen atom donation:
Compounds 1 to 5 act as electron donors, stabilizing free radicals and preventing further oxidative damage. The presents of conjugated double bonds facilitates resonance stabilization of radicals, reducing their reactivity.
3- Proposed mechanisms:
Antioxidant activity may occur through hydrogen atom transfer or single electron transfer reactions leading to neutralization of DPPH radicals and the formation of a stable product. Resonance stabilization of radical species further contributes to antioxidants effect.

Experimental results demonstrate concentration-dependent scavenging of DPPH radicals by compounds 1 to 5. Compound 4 exhibited the highest antioxidant activity scavenging DPPH radicals by 40% of the lowest concentration and 91% of the highest concentration. Compound 2 also displayed potent antioxidant properties with scavenging percentages ranging from 35% to 77% across different concentration. Compound 1, 3, and 5 showed moderate to strong antioxidant activity with the scavenging rates increasing with higher concentrations.

Overall compounds 1 to 5 demonstrated promising antioxidant potential (Figure 9), with compound 4 exhibiting the highest potency. These findings suggest that therapeutic utility of these compounds as natural antioxidants, offering potential benefits and mitigating deseases associated with oxidative stress.

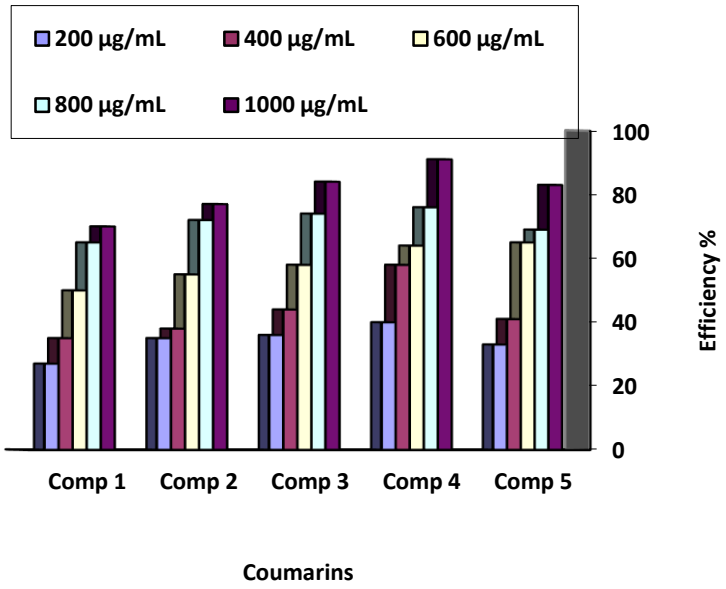

FIGURE 9 Antioxidant activities of various concentrations of compounds 1 to 5 against DPPH

Conclusion

This is study synthesized and assessed a series of coumarin derivatives for their antioxidant, antifungal, and antibacterial properties, revealing their potential as therapeutic agents in various medical applications.

The synthesized compounds exhibited significant antibacterial activity against both Gram-positive and the Gram-negative bacterial strains, with compound 4 demonstrating the most potent antibacterial effects against all bacterial species tested. Structural modifications such as the addition of nitrogen atoms and azomethane groups likely contributed to the enhanced antibacterial activity by increasing membrane permeability and disreputing bacterial cellular processes. Moreover, the compounds demonstrated antifungal properties with, compound 2 exhibiting notable inhibitory effects against various fungal species. The mechanisms underlying the antifungal activity may involve damaging fungal cell membrane disreputing cellular functions and including oxidative stress. The compounds also display remarkable antioxidant activity particularly compound 4 with showed comparable efficacy to ascorbic acid in savenging DPPH radicals. The antioxidant properties may steam from structural features of the compounds such as aromatic rings and functional groups facilitating hydrogen atom donation and radical reasonance stabilization. In summary since it to coumarin derivatives offer promising multifunctional capabilities as agents with antioxidant antifungal and antibacterial properties. Further research include *in vivo* assessments and structural activity relationship analysis is needed to fully understand their mechanisms of action and optimize their pharmacological properties for potential therapeutic applications.

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Authors' Contributions

Nadia Betti contributed to the conceptualization, methodology, and

investigation. Jaafa S. Shia contributed to the methodology, data curation, and formal analysis. Abdul Amir H. Kadhum contributed to the supervision, validation, and review and editing of the manuscript. Ahmed A. Al-Amiery contributed to the conceptualization, resources, project administration, and writing of the original draft.

Conflict of Interest

The authors declare no conflict of interest.

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